HIV & AIDS

Introduction

Human immunodeficiency virus (HIV) is the prototypical retrovirus. Retroviruses are characterized primarily by their RNA-dependent RNA polymerase, named reverse transcriptase. HIV-1 and HIV-2 represent just one subfamily of retroviruses. HTLV-1 and HTLV-2 represent another. They cause cancer. HIV-1 and HIV-2 are far more prevalent and way more relevant for your studies. HIV is fatal if not treated, spreads easily through various routes of transmission, and leaves its host organism quite well and able to spread infection for years. We ignore other subfamilies of retroviruses, keeping this lesson focused on HIV's pathogenesis, life cycle, and course of disease through AIDS and eventual death.

Transmission

The presence of **HIV in the blood, semen, and vaginal secretions** of infected people, and **the long asymptomatic period of infection** are factors that have promoted spread of the disease through sexual contact and exposure to contaminated blood and blood products. **Needles** and **sex** are what transmit HIV. Intravenous drug abusers (needles) and their sexual partners (sex), sexually active people (sex), and the newborns of HIV-positive mothers (birthed through the vaginal canal, so sex) are at highest risk for contracting disease. Tattoo needles are another potential means by which HIV can be transmitted.

The fetus and newborn are likely to acquire the virus from an infected mother. Transplacental infections are rare, but do occur. The highest risk to baby is during delivery. The mixing of infected vaginal secretions and blood during delivery may infect the neonate. C-section and active antiretrovirals can reduce vertical transmission.

Health care workers are at risk for HIV infection from accidental needlesticks and splash of contaminated bloods into broken skin or mucous membranes. Proper personal protective equipment should be used based on the procedure being performed. Seroconversion is rare, especially if postexposure prophylaxis is employed (see Viruses #8: *Antivirals*).

HIV is **not** transmitted by casual contact, touching, hugging, kissing, coughing, sneezing, insect bites, water, food, utensils, toilets, swimming pools, or public baths. You've got to mix blood, mother an infant, or have sex to get HIV.

ROUTES	SPECIFIC TRANSMISSION
Known Routes of Transmission	
Inoculation in blood	Needle-sharing among intravenous drug abusers
	Transfusion of blood and blood products
	Healthcare: Needlestick >> Mucous membrane splash
	Tattoo needles
Sexual transmission	Anal and vaginal intercourse
Perinatal transmission	Intrauterine transmission, peripartum transmission, breast milk
Routes Not Involved in Transmission	
Close personal contact	Any healthcare contact not blood
	Household members—sneezing, kissing, drinks

Table 6.1: Routes of Transmission

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HIV Virion

The HIV genome is technically a ss(+)RNA virus, so is single-stranded, made of RNA, icosahedral, and cytolytic, just like all other ss(+)RNA viruses (see lesson #4). The HIV virion is also **enveloped**. It must be enveloped because it brings with it proteins to let it do what it does—a polymerase, an integrase, and a protease. The polymerase is a special polymerase unique to retroviruses—**reverse transcriptase**. Reverse transcriptase is an **RNA-dependent DNA polymerase** which builds DNA from an RNA template—it reads RNA and makes DNA. The specific functions of these enzymes are discussed in the next section. The envelope has glycoproteins that allow for attachment and fusion, coded by the genome but not brought along with the envelope.

Genes are strings of nucleotides that code for mRNA. HIV has nine genes. Three of them are common to all retroviruses—*gag, pol,* and *env*—and are the ones to focus on. HIV is a complex retrovirus and has six other genes—*tat, rev, nef, vif, vpr, vpu*. We will explore what these genes do in the life cycle of HIV in the next section. Genes code for mRNA. That mRNA is turned into a string of amino acids. We use **gene product** to mean the unprocessed string of amino acids that is translated from mRNA. The gene product can then undergo post-translational modification, including cleavage. One gene product may subsequently be divided into several **proteins**.

For example, the *pol* gene encodes for four proteins. The *pol* gene's gene product has within it four proteins—protease, reverse transcriptase, integrase, and a protein you don't need to know about. The region of the *pol* gene that codes for the protease is termed *pro*; the region of the *pol* gene that codes for the reverse transcriptase, which is a polymerase, is frustratingly named *pol* (but is sometimes named *RT*); and the region of the *pol* gene that codes for integrase is named *int*. You must to be intimately familiar with the simple retrovirus genes used by HIV. We also spend a touch of time on those accessory genes.

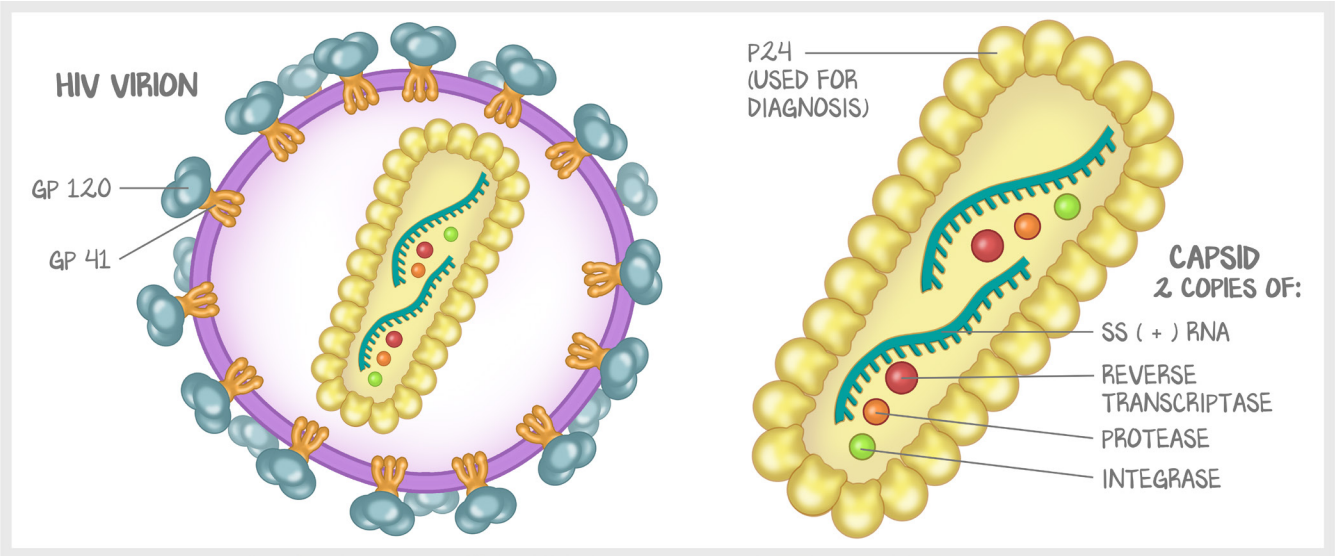

Figure 6.1: The HIV Virion
The HIV virion consists of envelope proteins, specifically the attachment glycoprotein gp120 and the fusion glycoprotein gp41. The capsid is lined by p24, which is used in screening for HIV infection. Both the antigen and the antibody to p24 are assessed in the screen. Within the capsid are two copies of viral genome (two viruses in one virion), reverse transcriptase, protease, and integrase.

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GENE	PROTEIN	NOTES
gag	p24	Capsid protein, HIV screen is based on Ab to p24
pol	pol	Polymerase: reverse transcriptase, protease, integrase
	int	Integrase, viral DNA inserted into host DNA
	pro	Protease, cleaves gene product of *pol* and *env* into proteins
env	gp120	Surface protein binds to CD4, CXCR4, CCR5
	gp41	Viral fusion with host cell
tat	tat	Transactivator, upregulates transcription of proviral DNA
rev	rev	Regulates viral RNA and mRNA fragments into cytoplasm
nef	nef	Decreases CD4 and MHC-I expression on host cells, manipulates T-cell activation pathways; required for progression to AIDS
vpr	vpr	Transport of complementary DNA to nucleus, arrest of cell growth

Table 6.2: HIV Genes to Proteins

HIV Life Cycle

HIV has been studied in incredible detail. So far, we've been very general in our discussion of how a virus replicates in a host cell. We've deliberately omitted the details of specific glycoproteins and molecular receptors from previous lessons. HIV is where we need to be super specific about which proteins are doing what. Such attention is paid because we know so much about it and because each step in the viral infection and replication cycle can be a target for therapeutics.

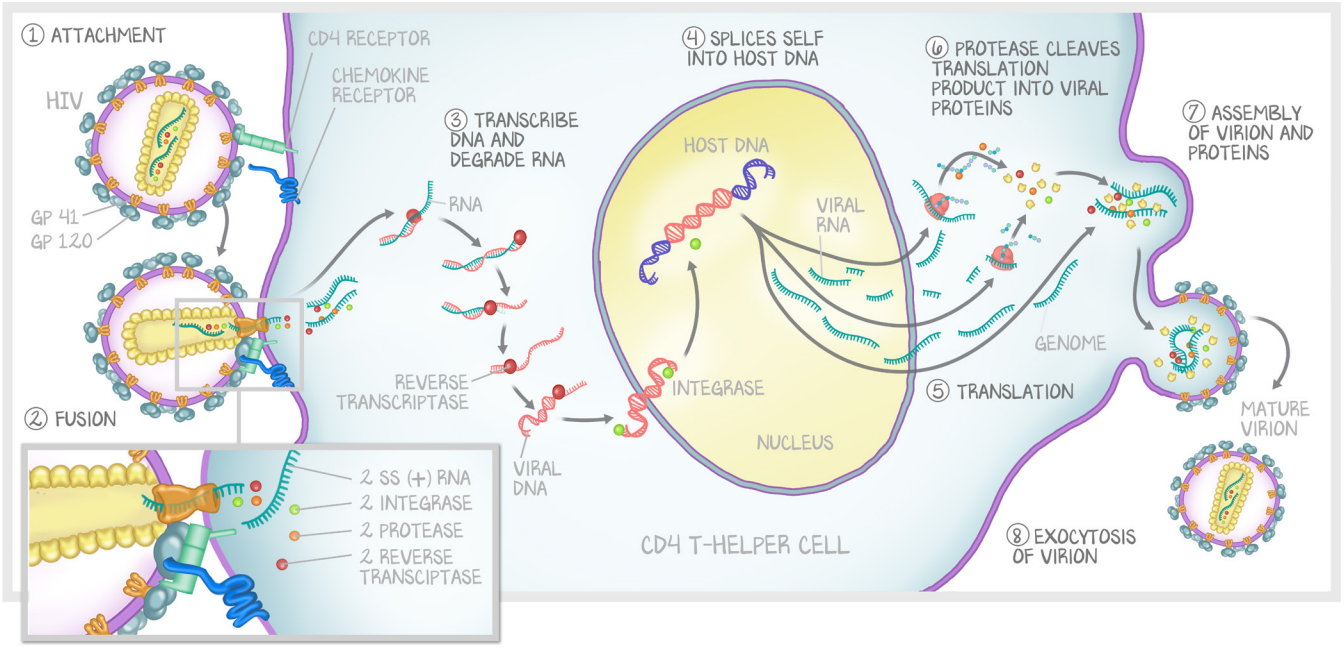

Figure 6.2: HIV Infection and Replication Cycle
This illustration accompanies the next two pages. The numbers in this illustration match the numbers in the text. Follow the text along with this image.

1. **Attachment.** HIV infects **CD4 T-helper cells** and **macrophages** because HIV can recognize surface proteins on these cells. **HIV gp120** (viral *g*lyco*p*rotein 120) of the virion envelope binds to **CD4** (host cell surface protein). HIV gp120 can also bind to chemokine receptors which act as coreceptors—**CCR5** and **CXCR4**. This attachment allows for gp41 to be deployed, and for entry of the virus into the cell.

2. **Fusion.** HIV is an enveloped virus. gp120 attaches the membranes together; activation of the chemokine receptor activates the cell and brings the membranes close together. **gp41** (glycoprotein 41) on the virion particle fuses the plasma membrane together, allowing the contents to be injected into the cytoplasm.

3. **Uncoating.** The mechanism of releasing the nucleocapsid into the cytoplasm after fusion and how the uncoating of the genome happens are not targets of therapies, and the mechanisms do not need to be known for testing or for clinical practice. We don't have any antivirals that target HIV's uncoating. Suffice it to say, the genomes, proteins, and transcription factors get in the cell.

4. **Reverse Transcription.** HIV has two copies of positive-sense single-stranded RNA. The virion **brings with it** an RdDp called **reverse transcriptase**. It copies both strands of ss(+)RNA, forming two identical RNA-DNA hybrid strands. That same enzyme then degrades the RNA strand, only to build a second **complementary** DNA strand, making **two double-stranded DNA** molecules the **cytoplasm**. From one virion, two ssRNA strands become two dsDNA strands, and the original ssRNA strands are gone.

5. **Integration.** The HIV virion **brings with it** an enzyme called **integrase**. The dsDNA and the integrase migrate to the nucleus. Once inside the nucleus, **integrase** integrates **viral dsDNA into the human genome** by splicing the viral dsDNA segment into the host DNA. Once integrated into the host genome, that stretch of genetic code is called a **provirus**. The host cell will now see that provirus as normal host DNA. So, it will be replicated if the cell divides, and it obeys all the regulations of gene transcription, which the provirus can use to its advantage.

6. **Transcription.** The regulatory proteins in the viral genome become active after integration—Tat, Rev, and Nef. The protein **tat**, made by gene *tat*, upregulates the rate of transcription. Tat is a cotransactivator which promotes the formation of the basal transcription unit, ensuring that the DNA-dependent RNA polymerase of the host cell attaches at proviral DNA. DdRp is regular host **RNA polymerase**, the enzyme of transcription. Transcription has two outcomes. If the entire stretch of provirus is transcribed, the product is **viral genome** (transcription is replication). If the virus uses transcription factors to transcribe only select genes, what gets transcribed is **mRNA for ribosomes**. The mRNA exits to the cytoplasm where ribosomes make the viral proteins. mRNA is allowed to exit the nucleus. Viral genome RNA doesn't have the post-transcription modifications necessary to get through the nuclear pore. The protein rev, made by gene *rev*, facilitates the transport of the viral RNA into the cytoplasm. The protein **nef**, made by gene *nef*, turns off expression of CD4 and MHC-I molecules, making the infected cell less likely to be spotted.

7. **Translation.** Unlike most ss(+)RNA viruses, the ss(+)RNA strands of HIV's genome are not read by ribosomes because they were destroyed by reverse transcriptase. Instead, **ribosomes read spliced viral genome**, the **mRNA** fragments that came from viral genome, to make the proteins HIV needs—the reverse transcriptase, integrase, and protease—as well as the structural proteins for the capsid and glycoproteins for the envelope. HIV is an extremely advanced virus—it does not rely on the host to code for any of its proteins. The amino acid sequence the ribosomes make isn't even what HIV wants. It won't let the host cell do the post-translational modification. HIV also brings with it **protease**, a protein that cleaves the product the ribosomes translate into the viral proteins HIV actually wants.

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8. **Assembly.** The virion is assembled—the ss(+)RNA strands, protease, integrase, reverse transcriptase, and structural proteins—then exits the cell coated in an envelope by budding off into cell membrane lined with viral glycoprotein.

Where HIV Goes and the Damage It Does

The primary target of HIV is the **CD4 T-helper lymphocytes**. While HIV does infect other cells, discussed below, the disease course is determined by the damage done to CD4 T-helper cells. HIV is cytolytic. Even though HIV is enveloped, integrates with the host DNA, and buds off as virions, CD4 cells die off. The CD4 count is normally between 800 and 1,000. If the CD4 count gets below 200, the patient is said to suffer from acquired immune deficiency syndrome (AIDS) and is at risk for opportunistic infections. The lower the CD4 count, the greater the risk. CD4 T-helper cells do not fight any infections themselves, but they are the connection between the innate and adaptive immune system. CD4 T-helpers are those Th0 cells from Immunology #10: *T-Cell Activation*. They listen to an antigen-presenting cell (APC), then, based on the type of antigen presented, differentiate into Th1, Th2, or Th17 cells, each of which expresses its own cytokines. Those cytokines are responsible for activating the adaptive immune system, as illustrated in Figure 6.3. **Without the CD4 T-helper, NO adaptive immune response can be generated.** B-cell isotype class-switching, B-cell activation of mature naive B cells, CD8 cytotoxic T-cell activation, neutrophil stimulation, macrophage stimulation, mast cell stimulation . . . literally every feature of the adaptive immune system is reliant on the CD4 T-helper to activate it. Without an adaptive immune system, the patient becomes at risk for **every infectious organism there is**—viral, bacterial, fungal, parasitic—and for **every cancerous cell** to become malignant—HCC, Kaposi's sarcoma, etc.

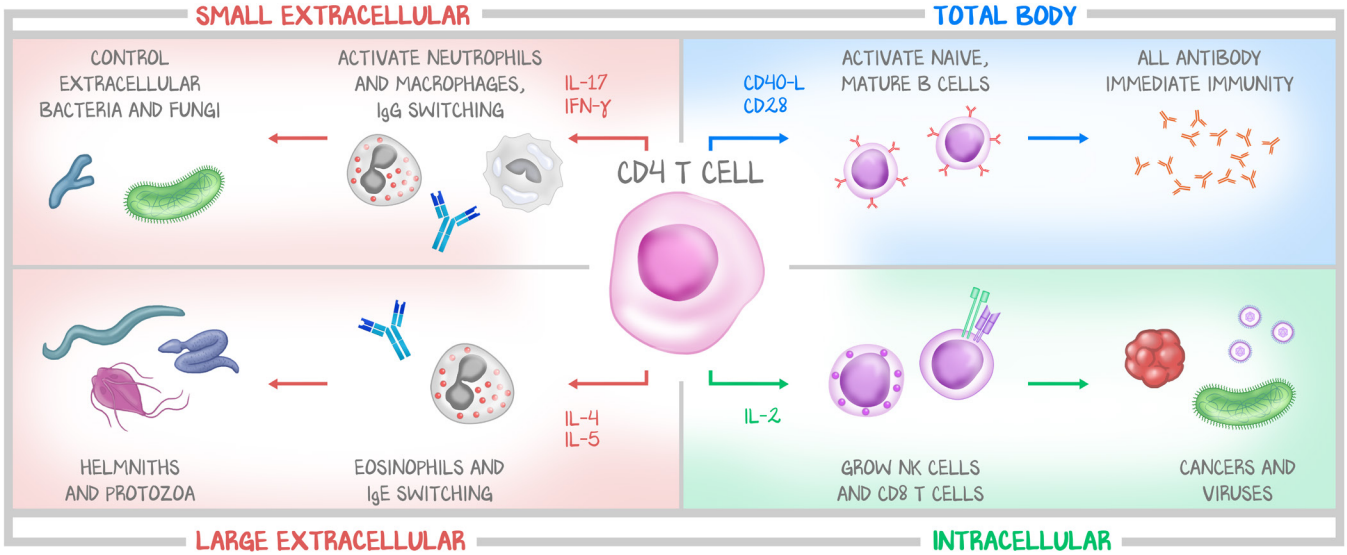

Figure 6.3: The Effects of CD4 T Cell Depletion
While HIV consumes only one cell type of immunity—the CD4 T helper cell—that one cell is the bridge between the innate and adaptive immune systems. It is responsible for activating phagocytes and IgG class-switching to fight bacteria (small extracellular). It is responsible for costimulation of mature naive B cells, which results in all antibody production of all isotypes. It is responsible for inducing CD8 cytotoxic lymphocytes to release cytokines and inducing apoptosis in virally infected or malignant cells (intracellular). It is also responsible for inducing eosinophils, mast cells, and IgE class-switching to fight parasites (large extracellular). In effect, the loss of CD4 T helper cells results in the total loss of immunity.

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Beyond the damage to CD4 T-helper cells and the impact that has on the immune system, other cells are affected. HIV itself causes a **wasting syndrome.** In Africa, wasting syndrome is called slim disease. Even with a well-controlled viral load, temporal wasting may never resolve, and that physical appearance carries with it stigma in communities highly affected by HIV (such as the gay community in America). **AIDS dementia** is caused by infection of the microglial cells in the brain, presenting as early-onset Alzheimer's symptoms, though the pathologic finding for Alzheimer's is not found on autopsy. **AIDS nephropathy** is caused by infection of the **epithelial cells** of the renal tubules, which can be further complicated by antiviral therapies, which also can cause renal impairment.

TYPE	SPECIFIC EXAMPLE
Protozoal	**Toxoplasmosis brain lesions**
	Cryptosporidiosis acute diarrhea
Fungal	***Pneumocystis jirovecii* (*carinii*) pneumonia**
	Candida esophagitis/gastritis
	Disseminated dimorphic fungi (blasto, histo, coccidio)
	Disseminated aspergillus
Bacterial	***Mycobacterium avium-intracellulare* Complex (MAC)**
	Extrapulmonary TB
	All pyogenic infections

TYPE	SPECIFIC EXAMPLE
Viral	Cytomegalovirus (CMV)
	Disseminated HSV
Cancers	**Kaposi's sarcoma (HHV-8)**
	HPV cancers (HPV)
	Hepatocellular carcinoma (Hep C, cirrhosis)
	Hodgkin's and non-Hodgkin's lymphoma (EBV)
Others	**AIDS dementia**
	AIDS nephropathy
	HIV wasting syndrome

Table 6.3: Examples of Opportunistic Infections

Natural Progression of Untreated Disease

On initial infection, the patient is unaware that it has happened. HIV uses CCR5 receptors to infect T cells in whichever tissue it was deposited into. People who have mutations of CCR5 are resistant to HIV infections. After about a two-week incubation period, the virus replication explodes, provoking the **acute illness phase**. The **viral load**, the quantity of HIV RNA, is extremely high (10^7 particles/mL of plasma). Antigens on HIV virions are also extremely high. One of the antigens on the HIV envelope that we can detect is **p24**. Either the amount of p24 or the presence of HIV RNA can be used to determine the presence of the virus during this time period. There are no antibodies to the virus yet, so detection of HIV antibodies cannot be used to detect infection.

This acute illness phase presents with a **mononucleosis-like reaction**—fever, lymphadenopathy, malaise—that self-resolves. During the acute illness phase, the host immune system recognizes the virus, and begins to form antibodies. The acute phase illness symptoms end.

Antibodies soar at day 30 following acquisition, and continue to rise until day 90. The presence of detectable antibodies is called **seroconversion**. Over the next 4–8 weeks (total 8–12 weeks from acquisition), antibody-mediated immunity and cell-mediated immunity destroy most of the virus. The viral load drops to 10^5 particles/mL of plasma. Destroying most of the virus causes a hit to the cells

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it infects, the CD4 cells. There is an acute drop in the CD4 count as infected cells are killed off. The CD4 count drops a few hundred points (normal is around 900, acute infection drops them to around 500). As the viral load plummets, the CD4 count rebounds (see Figure 6.4a). The viral load does not go to zero. The virus hides in T cells within lymph nodes. Replication continues and detectable virus is always present in the blood. Another acute phase, an explosion of viral replication, does not happen again, because antibodies are present. In the patient's blood are **HIV antibodies**, **HIV RNA**, and **HIV antigens** (this emphasis is relevant for screening, discussed below).

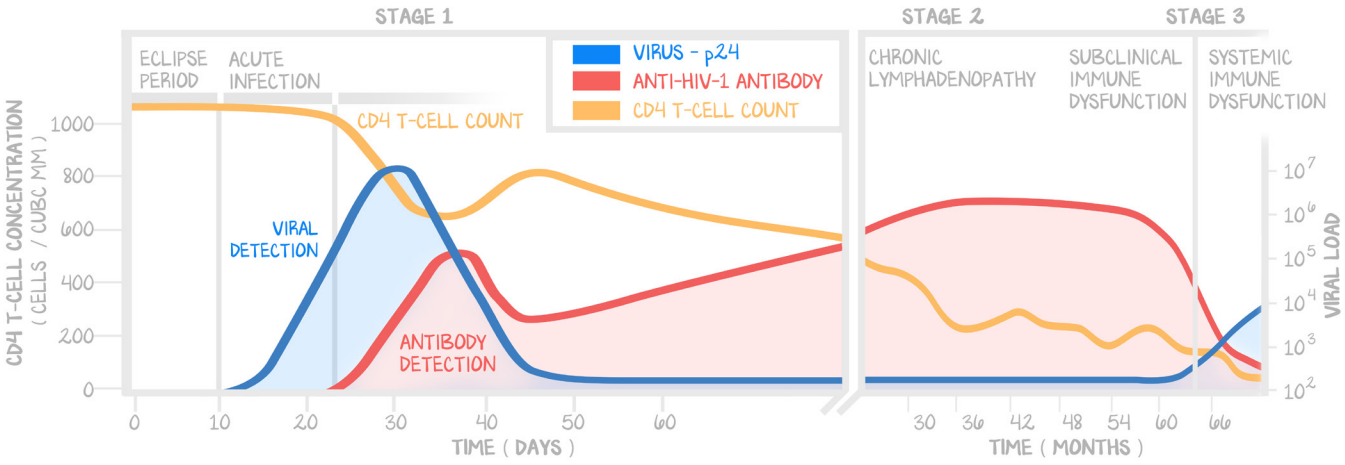

Figure 6.4: Acute and Chronic Infection Courses
Acute infection results in the loss of CD4 cells, but to a level that does not impair immunity. Acute infection ends with near-undetectable levels of virus. But the virus lives within CD43 cells. Over time, CD4 counts fall. They do so to varying degrees in different viruses and human genotypes, but for several years, on average, a patient is infected, infectious, and asymptomatic. The natural course ends with a loss of immunity, starting at a CD4 count of 200 (AIDS). In the final stages, all immunity is lost, antibodies plummet, and viral load begins to ascend.

The patient returns to completely asymptomatic. They are **infectious**. Over the next several years (there is enormous variation on how fast the deterioration occurs), the immune system continues to keep the virus subdued. But in keeping it subdued, it kills off infected CD4 cells. **CD4 count gradually decreases.** The patient becomes at risk for more frequent or more severe infections as the CD4 count drops below 500. But those infections are the infections that normally infect people—the patient does not notice simple, easily treated infections as a problem. Below a CD4 count of 200, AIDS. As the virus's preferred site of replication—the CD4 cells—begins to dwindle, the virus acquires a mutation that allows it a new target. Expression of CXCR4 increases, cellular tropism expands, and late-stage HIV becomes immune to fusion inhibitors (which target CCR5).

With a crippled immune system, the **viral load increases**. No longer kept subdued by antibody immunity, the viral load **gradually increases to acute-phase levels**. HIV RNA soars, p24 antigen soars. The virus is cytolytic. So even when no immune system is left, when there are no more CD8 cells to kill off infected CD4 cells, the virus still takes the CD4 count all the way to 0. The higher the viral load, the **more infectious the person**. In late-stage AIDS, the patient is the most infectious they have been since the acute phase.

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Making the Diagnosis

New Screening. The **best way** to screen is with the **fourth-generation antibody/antigen combination**. This is one test, given one time, that answers the question: are there HIV antibodies OR HIV antigen p24? Antibody detection works if antibodies are present—during the chronic infection phase. This captures most people undiagnosed and living with HIV right now. The combination of the antibody test with the p24 assay ensures that people newly infected (from the acute phase through the development of antibodies) and people without an immune system (at the very end of AIDS) will still be identified. If neither the antibody nor antigen is positive, the screening stops. If a positive screening is found, that same sample is screened with the **differentiation assay**, which separates HIV-1-Ab from HIV-2-Ab. If the differentiation assay is positive (two positive tests), the diagnosis is established, and **no further testing is required**. If the differentiation assay is negative, then you would do a nucleic acid amplification test **(NAAT)** looking for viral genome. We don't start with NAAT because it is expensive and is used as a confirmation test, not a screening test. p24 antigen is present in the patient from acquisition to death. Our assay cannot see p24 when HIV antibodies are present, because those antibodies bind to p24. This test combines a way to visualize p24 or HIV antibodies, effectively eliminating false negatives.

Old Screening. The old way of doing HIV screening and diagnosis was to **screen with antibody-only** and **confirm with Western blot**. Fourth-generation screens are expensive, so in resource-limited areas third-generation antibody-only-then-confirm is still a viable screening method. The limitation of the third-generation antibody screen is that because it tests only for the presence of antibody, it misses acute HIV infections AND it has a high false-positive rate. The Western blot is needed to confirm that the HIV protein—p24—is present.

Acute Retroviral Syndrome. With the new fourth-generation antibody/antigen testing, because of the presence of p24, even infections in the first few weeks after inoculation can detect **antigen**, a significant benefit over the antibody-only screens. However, the teaching remains that if someone has high-risk behavior, mono-like symptoms, and HIV is suspected, reverse transcriptase-polymerase chain reaction (**RT-PCR**) remains the preferred method of diagnosis. RT-PCR (the process) gives you the viral load (the number). Get a viral load, not the screen, for acute infection.

NAAT and PCR are essentially the same thing. An RT-PCR is a form of NAAT.

Treating HIV

HIV is treated with a combination of antiretrovirals, called HAART. The medications and treatment strategy are covered in Viruses #8: *Antivirals*. Here, we talk about the response to treatment as measured with the viral load and the CD4 count.

Viral load is detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Viral load determines the direction the patient is heading—lots of virus means the CD4 count will fall; no virus means the CD4 count will rise. Viral load also determines whether the medications are working (assuming patient adherence to the regimen). There should be a one-log decrease in the viral load in 2 weeks (measured at their 1-month follow up), and the viral load should go to undetectable in 3 months (measured at their 3-month follow up). A detectable viral load either means resistance has developed or the patient is nonadherent. Nonadherence to HAART is more dangerous than not taking it at all—resistance is bred quickly. **CD4 count** determines what the patient is at risk for right now (see above). CD4 changes by about 100 for every year of good therapy, and generally caps out at a recovery between 300 and 450 from whatever number the CD4 count was when therapy was started. Do not delay the initiation of HAART.

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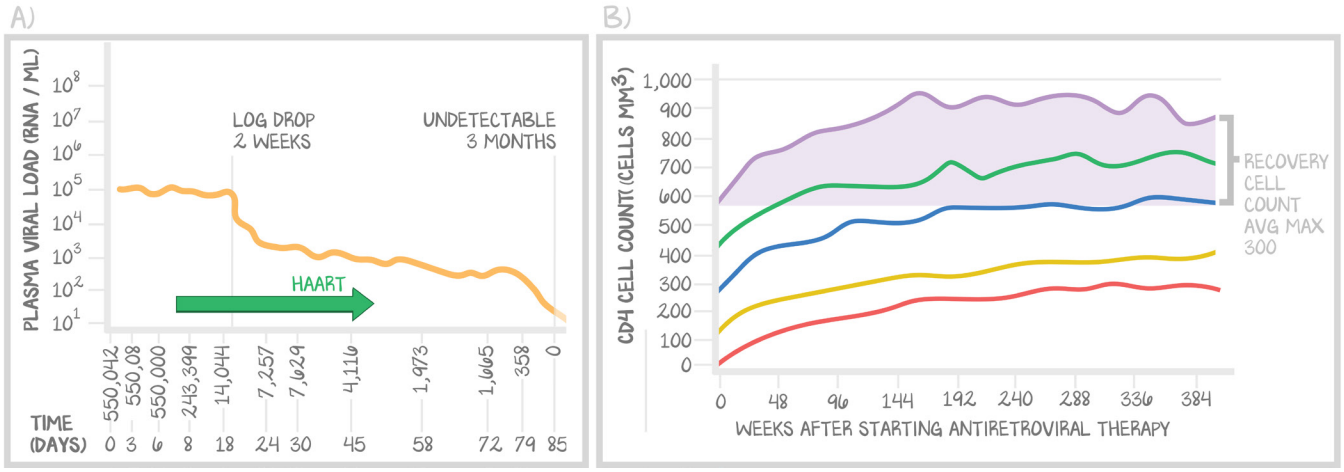

Figure 6.5: Natural Course of Treatment with HAART
(A) Response to HAART is brisk in regard to viral load. Within 3 months the patient has limited infectivity (though is still considered infectious), will no longer suffer a drop in CD4 count, and the virus should remain undetectable as long as the patient adheres to the medication regimen. (b) The response to HAART is slow and limited in regard to CD4 count. It takes years for the counts to recover. Most importantly, there appears to be a ceiling of recovery—the earlier the diagnosis is the made, the higher the CD4 count when HAART is started, the better the outcome.

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